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Lidocaine Injections Targeting CA3 Hippocampus Impair Long-Term Spatial Memory and Prevent Learning-Induced Mossy Fiber Remodeling

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Abstract

Learning a spatial location induces remodeling of the mossy fiber terminal field (MFTF) in the CA3 subfield of the dorsal hippocampus (Holahan et al., 2006; Ramirez-Amaya et al., 2001; Rekart et al., 2007a). These fibers appear to grow from the stratum lucidum (SL) into distal stratum oriens (dSO). Is this axonal growth dependent on ‘repeated and persistent’ neural activity in the CA3 region during training? To address this issue, we targeted local inactivation of the MFTF region in a post-training, consolidation paradigm. Male Wistar rats, bilaterally implanted with chronic indwelling cannulae aimed at the MFTF CA3 region, were trained on a hidden platform water maze task (10 trials per day for 5 days). Immediately after the 10th trial on each training day, rats were injected with lidocaine (4% w/V; 171 mM; n = 7) or phosphate-buffered saline (PBS; n = 7). Behavioral measures of latency, path length and thigmotaxis were recorded, as was directional heading. A retention test (probe trial) was given 7 days after the last training day and brains were subsequently processed for MFTF distribution (Timm’s stain) and cannula location. Lidocaine treatment was found to block the learning-associated structural remodeling of the MFTF that was reported previously and observed in the PBS-injected controls. During training, the lidocaine group showed elevated latencies and a misdirected heading to locate the platform on the first trial of each training day. On the 7-day retention probe trial, the lidocaine-injected group showed poor retention indicated by the absence of a search bias in the area where the platform had been located during training. These data suggest that reduction of neuronal activity in the CA3 region impairs long-term storage of spatial information. As this was associated with reduced MFTF structural remodeling, it provides initial anatomical and behavioral evidence for an activity – dependent, presynaptic growth model of memory.

Keywords

spatial memory; long-term retention; memory consolidation; mossy fibers; sprouting

Introduction

Brain lesion and pharmacological studies have highlighted the importance of the CA3 region for spatial information processing (Routtenberg, 1984; Kesner, 2007a; 2007b). Kainic acid lesions of the ventral CA3 region elevated latencies to locate the hidden platform during initial acquisition compared to sham-operated animals and also impaired reversal learning performance to locate the hidden platform when it was moved to a new location (Stubley-Weatherly et al., 1996). Kainic acid lesions of the dorsal CA3 region also impaired memory for the platform location compared to sham-operated rats while sham-operated and CA3-lesioned rats did not differ in acquisition performance (Roozendaal et al., 2001).

After preoperative training in a water maze the retention effects of cuts in the CA3 region that were oriented in the transverse plane were studied 7 days after surgery (Steffenach et al., 2002). Retraining took place in a different room. Sham-operated animals showed a search bias for the platform location 7 days after surgery while rats with transverse cuts in the CA3 region failed to search preferentially in the target quadrant. Retention was preserved after partial, as opposed to complete “fiber-sparing” lesions of CA3 pyramidal cells after ibotenate injections, suggesting that retention was primarily determined by fiber disruption rather than limited CA3 cell loss.

Chelation of zinc from mossy fibers in the dorsal CA3 region with diethyldithiocarbamate (DDC) prior to learning impaired the acquisition of spatial information yet performance measures were similar to controls indicating no sensorimotor deficits (Lassalle et al., 2000). A related study found that the DDC-impairing effect was selective for spatial but not cued, non-spatial water maze tasks (Florian and Roulet, 2004). In a spatial object recognition task, when injections of DDC were given into the CA3 before or immediately after training, information processing was impaired; when injections were given before the retention test, no deficit was observed (Stupien et al., 2003).

There is increasing evidence that the MFTF may regulate spatial learning and memory by remodeling its synaptic connections. Previous work has shown that spatial water maze training is associated with a larger hippocampal mossy fiber terminal field (MFTF) in CA3 distal stratum oriens (dSO) of adult Wistar (Holahan et al., 2006; Ramirez-Amaya et al., 2001; Ramirez-Amaya et al., 1999; Rekart et al., 2007a; Rekart et al., 2007b) and Long Evans rats (Holahan et al., 2006). This effect is specific to hidden platform spatial memory tasks as cued, visible platform learning does not promote such growth (Rekart et al., 2007a). Because neural activity in the granule cell-mossy fiber-CA3 circuit appears to encode spatial memory (Jung and McNaughton, 1993), the present study determined whether blockade of such activity in this circuit would impair spatial information processing and learning-associated MFTF remodeling.

Materials and Methods

Subjects

Subjects were 14 male, Wistar rats (Charles River) that weighed 300 - 350 g at the start of the experiment. They were housed in pairs with free access to food (Purina rat chow) and tap water. The temperature (22° C) and light/dark cycle (lights on at 6:00 am; lights off at 6:00 pm) of the vivarium were controlled. Animal care conformed to guidelines of the National Institute of Health Guide for the Care and Use of Animals and animal use protocols approved by the Northwestern University Animal Care and Usage Committee.

Surgery

Rats were anaesthetized with sodium pentobarbital (50 mg/kg) and chronically implanted bilaterally with guide cannulae aimed above the CA3 region of the dorsal hippocampus. Using standard stereotaxic techniques with the tooth bar set at - 3.5 mm (Paxinos and Watson, 1998) the guide cannula tips (26 ga; 8 mm) were placed at coordinates (in mm from bregma and skull) AP: -3.1, ML: -3.0, DV: -2.5. Cannulae were anchored to the skull with two screws and dental cement and kept patent with removable stylets. Following surgery each rat was given 0.3 ml penicillin (300,000 u) and placed into a heated holding cage. After recovery from anesthesia, the rats were given a subcutaneous injection of Flunixin and allowed to recover for one week before the behavioral procedure began.

Apparatus

The water maze was a black-painted stainless steel container 2 m in diameter, 60 cm high and filled to a depth of 35 cm with 22° C clear water. The platform (10 cm diameter) was 1 cm below the surface of the water and was made from black plastic. The pool was located in a test room one floor above the animal housing room. The test room was 3 m × 4 m, illuminated by 4 spotlights aimed at the ceiling. Within the room there was a black radial arm maze leaned against the north wall (not true compass direction), a cabinet with a black circle on the west wall, a white tapestry on the east wall with a black triangle, a video camera mounted on a tripod located at the northeast end of the pool, a radio located at the southwest end of the pool and a desk at the south end of the pool on which a computer (monitor and CPU) were located. The experimenter sat in front of the computer and was visible from the pool. The door to the room was located opposite the experimenter. Swimming behavior was tracked using HVS software.

Water maze training Days 1--5

Animals were handled for 5 min on each of 4 days before beginning water maze training. Training on the hidden platform water maze task occurred over 5 days with 10 massed trials per day. On each trial, a rat was placed into the pool at a different point on the perimeter (pseudorandomly selected). The hidden platform was located in the middle of the northwest quadrant of the pool throughout the 5 days of training. On each trial, a rat was allowed to swim freely for up to 60 sec; if a rat did not find the platform within this time, the experimenter guided it there. The rats remained on the platform for 30 sec after the end of each trial. Each rat was then taken off the platform and placed into a holding container for an additional 30 sec.

Rats swimming behavior was tracked using the HVS Image 2100 Tracking System. Using this technology, two dependent measures were analyzed in this report including: 1) latency, the amount of time in seconds (s) required to swim from the launch point on the perimeter of the pool to the platform; 2) heading, or start-angle, defined as the start-direction relative to the ideal direction measured in degrees (ideal being 0°). HVS gives values relative to ideal, which includes both positive and negative values. A positive value is calculated as the heading taken from ideal in the clockwise direction while a negative value is calculated as the heading taken in the counter clockwise direction from ideal up to a max value of 180° in both directions; therefore, taking absolute values removed a significant amount of variation. For analysis, absolute values were obtained (negative values were converted to positive).

Injections

Immediately after the 10th trial of each training day, rats received an injection of lidocaine hydrochloride (4% w/v in 0.01 M phosphate-buffered saline, PBS; n = 7) or 0.01 M PBS (n = 7). Animals were assigned to each group randomly. The drug was mixed each day. Drug

or vehicle was back-filled into the injectors (32 ga stainless steel tubing that extended 1 mm beyond the guide cannulae) with a 10 μ l Hamilton syringe connected to the injector via PE 10 plastic tubing. For each injection, the animal was removed from the water maze and brought into a separate room where the injection system was located. The experimenter held each animal during the injection. Injections were given in a volume of 0.5 μ l injected at a rate of 0.1 μ l/ min followed by a 2 min diffusion period. Each animal was given 5 total injections (1 post-training injection after the completion of training on each day).

Water maze retention – Day 12

Seven days after the fifth training day, the animals were given a probe retention test. There was no platform in the pool on this day and rats were not injected. The launch point for the probe trial was randomly chosen for each animal. The probe trial lasted 30 sec (see Blokland et al., 2004) and is considered to measure the strength of spatial learning retention (Cassel et al., 1998).

Measures were taken from each animal on the amount of time spent searching near the original platform location in the target quadrant and three other randomly selected locations in the other quadrants. The region of interest used was an annulus 4 times the platform diameter. The 40 cm diameter annulus area (1,256 cm²) covers 4.0% of the total pool area (31,400 cm²). We believe this region of interest more accurately reflects spatial preference because rats are searching in an area closely associated with the platform location. The circular region of interest neglects time spent along the wall, which likely does not reflect an informed search of a previous platform location (Blokland et al., 2004).

Histology

Immediately after the probe test, animals were individually removed from the water maze and given an overdose of sodium pentobarbital (Nembutal; 50 mg/kg) followed by transcardiac perfusion with 0.9% saline. Brains from each animal were prepared for Timm's staining by placing them into 1% sodium sulphide at room temperature for 20 min then transferring them to a 3% glutaraldehyde/ 4% paraformaldehyde/ 0.1 M phosphate buffer solution and storing them overnight at 4°C. The following day, brains were allowed to submerge in a 30% sucrose/ 0.1 M phosphate buffered solution.

Brains were sectioned on a cryostat at 40 μ m and collected in 0.1% sodium azide/0.1 M phosphate buffer. Brain sections were processed using a Timm's stain procedure as previously described (Cantalops and Routtenberg, 1996).

Timm's quantification

Unbiased areal measurements were carried out in the manner described by Holahan, et al., (2006). Using an Olympus BX61 microscope with a DP70 Olympus camera (12.5 megapixels), digital images of the dorsal hippocampus were obtained (10x, NA 0.4). A grid (squares = 25.0 μ m/side) was randomly placed over an area of the dorsal hippocampus defined medially by the lateral tip of the granule cells of the infra- and suprapyramidal blades and laterally by the fimbria. Two coronal sections (140 μ m between sections) were sampled from the dorsal hippocampus starting 200 μ m from the initial blossoming of the hippocampal CA3 pyramidal cells (approximately -1.8 mm from bregma) prior to visualization of the cannula tracts. Counts for each region of interest were taken as averages from both coronal levels. The areas of the *stratum lucidum* (SL) and the distal *stratum oriens* (dSO) were estimated by multiplying the number of points on the grid overlaying a Timm's-positive granule in each region by the area per point. A Timm's-positive granule was defined as having a gray value twice that of the background as measured on the *stratum radiatum* (SR) of the CA3 region. Comparisons between groups were made using the ratio

of the area in dSO to that in SL to account for size variations between individual animals. An experimenter who was blind to group assignment carried out all analyses.

Results

1. Histology: Cannula placement in relation to MFTs

Figure 1 shows the histological sections at the level of the cannula implantations. Injection sites are 1 mm below cannula tip placements and are shown for the lidocaine group (Figure 1A) and for the PBS controls (Figure 1B). Injection sites were located within the CA3 region terminating in the *stratum lucidum* (SL) or located in the more dorsal aspect of the CA3 region encroaching on the *stratum pyramidale* (SP). Also of note in these sections, Timm's staining of MFTs was evident lateral to the injection site. This indicates that even when the cannulae or vehicle injections partially transected the MFT in the more medial aspects of the SL layer, there was no discernible reduction in Timm's staining indicating the prevalence of intact MFT axonal terminals. In sum, target locations of implanted guides and injectors were proximal to the MFT with no apparent difference in the distribution of lidocaine and vehicle injection sites.

2. Learning-induced alterations in Timm's staining: effect of lidocaine

Using a point-grid analysis (see Methods in current report and methods in Holahan, et al., 2006), the MFT-stained area was quantitatively studied using two coronal sections (see Methods in current report for selection process) from each animal. Counts for each region of interest were taken as averages from both coronal levels (Figure 2A). Comparisons between groups were made using the ratio of the area in dSO to that in SL as in Holahan, et al., (2006; see Figures 3 and 6 in that paper for a comparison). The lower the ratio the less the remodeling. In prior studies naïve Wistar rats have a ratio of 0.20 or below, while trained Wistar rats reach ratios of 0.40 or higher (Holahan, et al., 2006).

The PBS-control group showed learning-associated remodeling of the MFT, indicated by the ratio of 0.406 ± 0.055 (mean $\pm$ SEM) for the dSO:SL while the lidocaine group showed little, and at times no, apparent learning-associated remodeling of the MFT as indicated by the 0.064 ± 0.015 dSO:SL ratio for the lidocaine group (Figure 2B). This larger expansion in the controls was confirmed by an independent samples t-test (Figure 2C) revealing a significant difference ($t(12) = 5.98$, $p < 0.01$) between the lidocaine and PBS groups.

Interestingly, the ratios did not depart significantly from our prior studies in which no cannulae implantation was carried out (Holahan, et al., 2006). In non-operated Wistar rats trained on the hidden platform water maze task, the mean dSO:SL ratio was 0.41; not significantly different from the PBS-injected group ratio of 0.47 (see Table 1 for individual dSO/SL ratios; t-test, $p = 0.32$). This suggests that multiple injections of PBS had little effect on the spatial-learning induced MFT remodeling.

3. Latency

As shown in Figure 3A, during the 5 days of water maze acquisition, while the latency to locate the platform on Trial 1 of Days 2 through 5 decreased in the PBS group, there was no similar latency decrease in the lidocaine-injected group. Both groups did show decreasing latencies within an individual day. Latency data were analyzed by taking the difference in time required to locate the platform on Trial 1 and that required to locate the platform on Trial 10 of the previous day (e.g., Day 2, Trial 1 latency minus Day 1, Trial 10 latency). As can be seen in this analysis plotted in Figure 3B, the PBS control group showed a significant decrease in this measure over time going from a high positive value (indicating little savings from Trial 10 to Trial 1) to a negative value (savings indicated by a lower latency to locate

the platform on Trial 1 of Day 5 than that required to locate the platform on Trial 10 of Day 4). The lidocaine group, on the other hand, did not show this pattern and in fact, demonstrated no improvement over time. A two-way repeated measures ANOVA (group by time) revealed a significant interaction between these two factors ($F(3,36) = 4.04$, $p < 0.02$) with a significant difference occurring between groups on the Day 4/Day 5 comparison ($p < 0.05$). This suggests that the lidocaine-group showed little savings from Trial 10 to Trial 1 over days.

A further sub-analysis was carried out on the lidocaine group's search pattern on Trial 1 of Day 5, when they showed longer latencies than the PBS group. Measures were taken from each lidocaine-injected animal on the amount of time spent searching near the platform location and three other randomly selected locations in the other quadrants. The region of interest used was an annulus 4 times the platform diameter (similar to that used for the 7-day probe test). Figure 3C shows that during this trial, the lidocaine-injected group did not spend time searching in close proximity to the platform but rather spent significantly more time searching in the adjacent right quadrant ($F(3,24) = 6.03$, $p < 0.01$; adjacent right versus all other platform locations, $p < 0.01$, Fisher's least significant difference post hoc tests). This indicates that the lidocaine group showed very little 24-hour recall of the platform location after 4 days of training.

4. Path Direction from Start to Platform ('Heading')

The lack of 24 h savings was also seen in a different measure, which was latency-independent. The ideal direct path from the start location to the platform makes an angle of 0° (straight towards the platform). Any deviation from this perfect targeting is computed automatically by the HVS software and reported in degrees as heading. These data are shown over the 5 days in Figure 4A. The heading taken by the PBS control group approached 0° over the 5 days. In contrast, the lidocaine group appeared to show no improvement in the direction taken to locate the platform over this 5-day training period. Heading data were analyzed as the absolute heading taken to locate the platform on Trial 1 for each day (Figure 4B). The absolute heading for the PBS control group approached 0 by the end of training period while the lidocaine group showed little difference between the heading recorded on the first trial of the first day and that recorded on the first trial of the last day of training. A two-way repeated measures ANOVA (group by time) revealed a main effect of group ($F(1,12) = 17.96$, $p < 0.01$).

5. Probe test

Seven days after the fifth training day, and 7 days after the last injection, the animals were given a retention test. There was no platform in the pool on this day and rats were not injected. Figure 5 shows the data presented as percent time in each quadrant in relation to an annulus 4 times the platform diameter. The 40 cm diameter annulus area ($1,256 \text{ cm}^2$) covers 4.0% of the total pool area ($31,400 \text{ cm}^2$) so random search behavior would be less than 5%. Annulus data were analyzed with a two-way repeated measures ANOVA (group by annulus location). This revealed a significant interaction between factors ($F(3,36) = 3.13$, $p < 0.05$) with the PBS group spending more time searching near the previously located platform region than the lidocaine group ($p < 0.05$).

Discussion

Although it is tempting to think that the observed reduction in mossy fiber (MF) growth leads to the impairments of long-term memory on the probe test, it is important to be clear that even if the disruption were shown to be restricted to only the MF synapse, no implication is made that the memory is stored at this synapse. Rather, memory is impaired

because the network, in which the MF synapse is embedded, is disrupted. Thus, MF synapse disruption, even hypothetically, is the consequence of interfering with a key communication point in a larger network. Our perspective is that the present study represents a novel starting point for investigating how networks are formed after learning occurs; the focus point on MF synapses is thus an opportunity for gaining fresh insight into a fundamental issue that relates structural change to memory formation.

Two retention deficits were observed after post-training bilateral lidocaine (but not vehicle) injections into the CA3 region of the dorsal hippocampus. First, 24 hours after the injection, latency to reach the hidden platform was elevated. There was also a misguided start-direction relative to the ideal ('heading'). The second deficit was observed 7 days after the last training day during a probe test when the lidocaine-injected group showed no preference for the previously learned location of the platform. It is suggested that the 24 h impairment is based on disruption of post-trial cellular events, whereas the 7-day impairment may, in part, be ascribed to blockade of long-lasting changes in growth, including but not limited to, mossy fiber terminal field (MFTF) remodeling.

Lidocaine, which blocks sodium channels for 15 – 30 minutes (Martin, 1991; Pereira de Vasconcelos et al., 2006), was given immediately after training each day, thereby disrupting a process that occurs within the first 30 min after training, consistent with a memory consolidation mechanism (see McGaugh, 2000 for review). The present results do not identify the target(s) for this blockade but likely candidates include granule cell inputs via mossy fibers CA3 axon collaterals, dendrites and thorny excrescences, inhibitory interneurons associated with the mossy fibers (Vida and Frotscher, 2000) or CA3 pyramidal cells and their axonal projections to CA1.

The lidocaine injections given post-trial during the 5 day learning period, blocked both retention 7 days later as measured during a probe test and MFTF remodeling (at rostral levels of the CA3 dorsal hippocampus) seen here in vehicle controls and in prior studies (Holahan et al., 2006; Rekart et al., 2007a; Rekart et al., 2007b). Other structural remodeling after learning may also occur within the SL itself (Gogolla, et al., 2009), within the elaborate axonal network of inhibitory interneurons that is intercalated with the MFTF in the SL (Vida and Frotscher, 2000).

Lidocaine injections into the CA3 region likely also interfere with CA3 output to CA1 via the Schaffer collateral pathway. Previous work has shown that administration of lidocaine into the CA1 region prior to water maze training impairs acquisition (Bohbot et al., 1996; Parron et al., 2001; Riekkinen et al., 1999). As well, CA1 lidocaine injections before the probe retention test impaired the ability of rats to demonstrate spatial learning (Broadbent et al., 2006; Pereira de Vasconcelos et al., 2006). This suggests the possibility that post-training lidocaine injections into the CA3 region that disrupt spatial information consolidation might involve disruption of CA1 pyramidal cell function (but see Bohbot et al., 1996).

Intra-CA3 post-training lidocaine injections may also be effective in impairing memory processes by disrupting granule cell output functions. In this regard, the contribution of neurogenesis to learning-associated remodeling of MFTFs may be considered as hippocampal-dependent learning enhances the survival of adult-derived granule cells (Gould et al., 1999). However, a comparison of the rapidity with which learning-associated presynaptic remodeling is observed in Long Evans rats (Holahan et al., 2006) with the time required for granule cell axonal extension (Hastings and Gould, 1999), together suggest that it is the remodeling of existing terminals, rather than axons of newly minted granule cells, that contributes to learning-associated MFTF remodeling. In this regard, studies of 'local

regulation' of mossy fiber terminal plasticity involving protein kinase C signaling mechanisms may be especially relevant (Galimberti, et al., 2006).

The present results call attention to the critical role that MF presynaptic mechanisms are likely to play as part of the neural substrates underlying long-term mnemonic function. The re-distribution of presynaptic MFTFs described previously appear to depend on patterned neural activity within this system (Jung and McNaughton, 1993). Such facilitation could arise as a consequence of shifting input away from inhibitory interneurons (Aradi and Maccaferri, 2004; Maccaferri et al., 1998; Mori et al., 2004; Vida and Frotscher, 2000).

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Figure 1.

Injector placements in relation to mossy fiber terminal field. Brain sections from (A) the lidocaine and (B) vehicle (PBS) control groups were processed for the Timm's heavy metal stain and counterstained with cresyl violet. A representative section from each animal in each of the two groups is shown. Scale bars (in lower right corner) = 500 μm.

Figure 2. (A) Mossy fiber terminal field (MFTF) staining was quantified using a point-grid analysis as described in the Methods section. (B) Upward arrow on the histology image from a PBS-injected animal indicates Timm's staining in *stratum oriens* (dSO; 20x magnification). This staining is absent in the lidocaine-injected histological image (downward arrow). (C) Quantification of the dSO/SL ratios. Ratios are expressed as the mean ratio ($\pm$ SEM) of the stained area in dSO to the mean area in *stratum lucidum* (SL). This analysis showed that post-training lidocaine treatment during training blocked the MFTF remodeling to dSO that was observed in the PBS-injected group; **, $p < 0.01$ PBS vs. lidocaine.

Figure 3.

Latency measured in seconds to locate the hidden platform. Data are expressed as means $\pm$ SEMs. (A) Latencies were plotted for individual trials (10 trials/day) over the 5 training days. Injections (arrows) were given immediately post-training each day. Although 24 h retention was impaired, both lidocaine ($n = 7$) and PBS ($n = 7$) groups showed decreased latencies to locate the platform within a given day. (B) Latency differences were computed from the latency on Trial 1 for days 2 - 5 and the latency from Trial 10 for days 1 - 4 and plotted as day pairs (e.g., d1d2 represents latency for Trial 1 on Day 2 minus latency for Trial 10 on Day 1). Statistical analysis revealed a significant days by trials by group interaction: the PBS-injected group showed shorter latencies over days on Trial 1 compared to Trial 10 of the previous day while the lidocaine-injected group showed no improvements over the course of training ($* p < 0.05$ PBS vs. lidocaine on the d4d5 data). (C) Search pattern on Trial 1 of Day 5 in the lidocaine group. Measures were taken on the amount of time spent searching near the platform location (target; 4 times the platform diameter) and three other randomly selected locations in the other quadrants. The lidocaine-injected group did not spend time searching in close proximity to the platform indicating that this group showed very little 24-hour retention for the platform location after 4 days of training ($**p < 0.01$, time spent in adjacent right (adjrt) annulus versus all other annuli. Abbreviations for this and Figure 5 are: adjrt = quadrant to the right of the target quadrant, oppo = quadrant opposite to the target quadrant, adlft = quadrant to the left of the target quadrant and target = where the platform was located during training.

Figure 4.

The initial directional heading taken to locate the hidden platform was computed automatically by the HVS tracking software. Headings in (A) are expressed as the mean $\pm$ SEM for each trial of the recorded deviation, as measured in degrees, from the ideal heading (0°). The ideal heading is a straight line from the launch point to the platform. The lidocaine group showed a dramatic deviation from the ideal on the first trial of each training day. Data in (B) are expressed as the absolute heading for Trial 1 of each training day. Data were converted to absolute values to reduce variance. Over the 5 training days, the PBS group approached the ideal heading on Trial 1 whereas the lidocaine-injected group did not. Analysis revealed a main effect of group (* $p < 0.05$, PBS vs. lidocaine) on the Trial 1 data.

Figure 5.

A probe retention test was given 7 days after the last day of training. There was no platform present and no injections were given. Data are expressed as mean $\pm$ SEM for the percent time spent in an annulus 4x the diameter of the platform. Line on figure indicates chance performance. * $p < 0.05$ percent time spent in the target annulus, PBS vs. lidocaine. Abbreviations as in Figure 3.

Table 1

Individual data for the dSO/SL ratios from the present study and those found in Holahan, et al., (2006) as indicated with *. Ratios are expressed as the mean ratio ($\pm$ SEM) of the stained area in distal *stratum oriens* (dSO) to the mean area in *stratum lucidum* (SL). Of particular interest is the similarity in ratios found in the lidocaine-injected and yoked control groups and the similarity in ratios between the PBS-injected and hidden-trained groups.

	lidocaine	yoked controls*
1	0.078	0.173
2	0.072	0.175
3	0.035	0.110
4	0.026	0.303
5	0.125	
6	0.020	
7	0.091	
mean	0.064	0.190
SEM	0.015	0.031
	PBS	hidden-trained*
1	0.465	0.406
2	0.485	0.480
3	0.558	0.526
4	0.506	0.465
5	0.188	
6	0.428	
7	0.212	
mean	0.406	0.469
SEM	0.055	0.019